University of Kentucky

IDENTIFYING BISPHOSPHONATE PROTEIN BIOMARKERS IN EQUINE SERA USING MASS SPECTROMETRY METHODS

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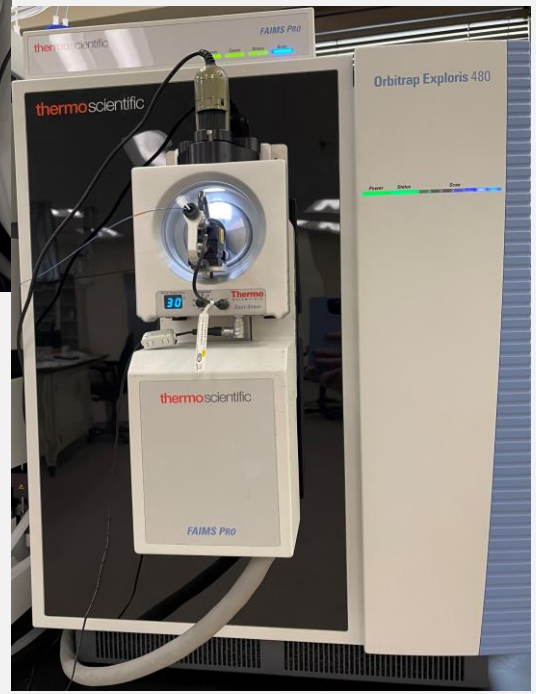
INTRODUCTION

- Bisphosphonates are a class of drugs that have not been extensively studied in equine but have been used in humans for several years

- Prohibited for use in racehorses
- Importance of drug testing in performance horses
  - Designer drugs
  - Rapid elimination times (e.g., lack of metabolism of bisphosphonates)
- Monitoring protein effects allow for larger detection windows compared to small molecule methods of drug identification

METHODOLOGY

- Same for discovery and PRM methods
- Serum collected from in-training equine subjects following Tildren® administration over a monitored time course
- Albumin depletion performed using organic solvent fractionation (IPA/TCA)
- Depleted sera was enzymatically digested using trypsin for LC-MS/MS analysis
- Data-dependent analysis on a Thermo Orbitrap Exploris 480 coupled with an Ultimate 3000 RSLC nano (Thermo Scientific™)
- Performed peptide, protein, and label-free quantitation



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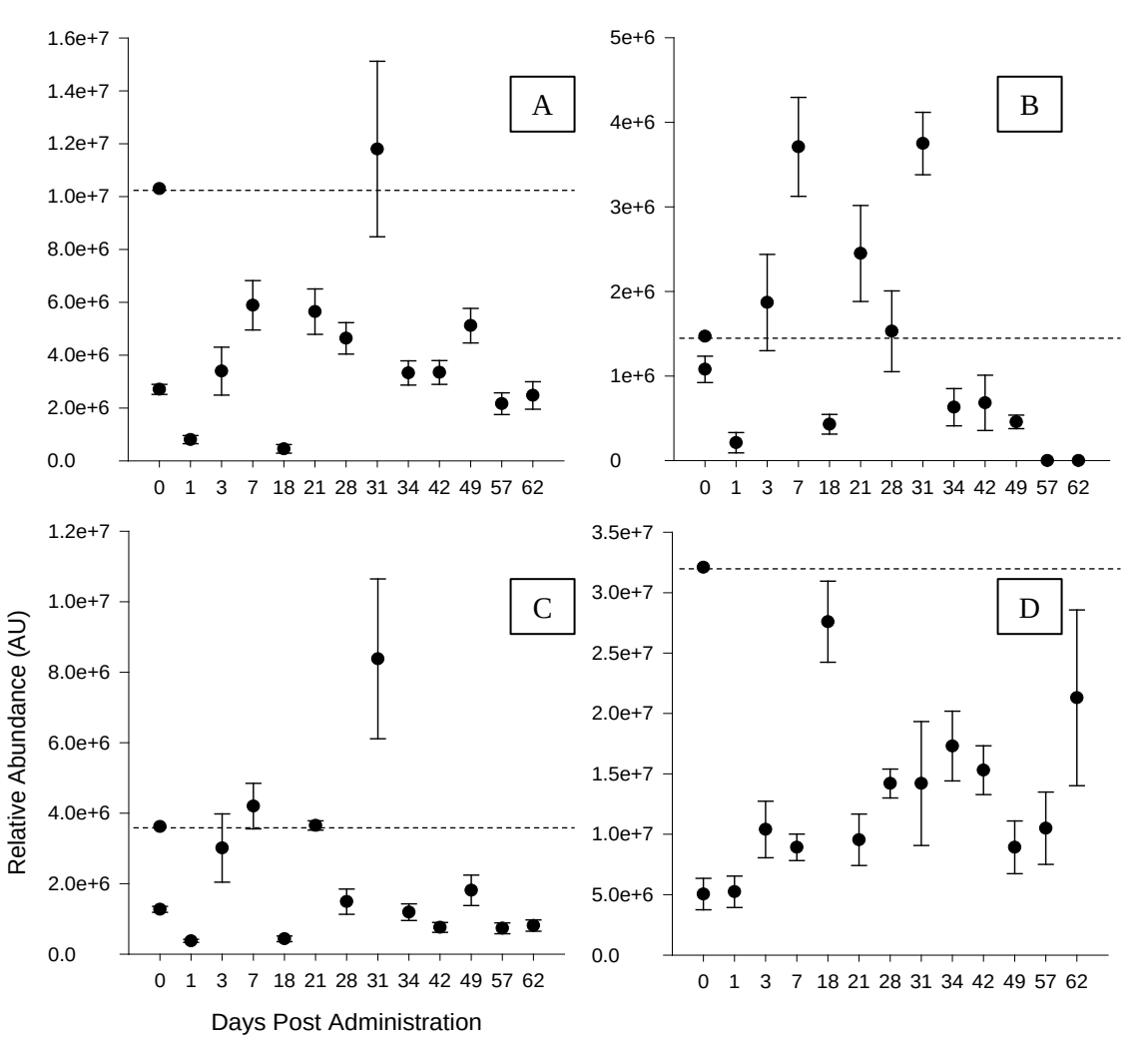
DISCOVERY RESULTS

- Identified proteins from discovery data
  - Actin, cytoplasmic
  - Carbonic anhydrase
  - Fibrinogen
  - Fibronectin

Protein	Accession No.
Actin Beta Like 2	F7AHF3
Actin Gamma 2, Smooth Muscle	A0A3Q2HBP5;A0A3Q2KTQ9
Actin, Cytoplasmic 1	A0A3Q2KTQ9
Carbonic anhydrase	A0A3Q2HMG5; F6ZBG0
EGF Containing Fibulin Extracellular Matrix Protein 1	F6PVG3
Elongation Factor 1-alpha	F6UME7
Extracellular Matrix Protein 1	F6QYS3
Fibrinogen	A0A3Q2GS38
Fibrinogen Alpha Chain	A0A3Q2HTG2
Fibrinogen Gamma Chain	A0A5F5PPB8
Fibrinogen Beta Chain	F6PH38
Fibronectin	A0A5F5PTE1; F7CN11; Q28377
Fibulin 1	F7ABC9
Intercellular Adhesion Molecule 3	F6SIE2
Interleukin 1 Receptor Accessory Protein	A0A5F5PI46
Matrix Metallopeptidase 19	F7C6S0
Talin 1	F6QIZ4
Transforming Growth Factor-Beta-Induced Protein ig-h2	F6UMQ4
Vitronectin	F6V881

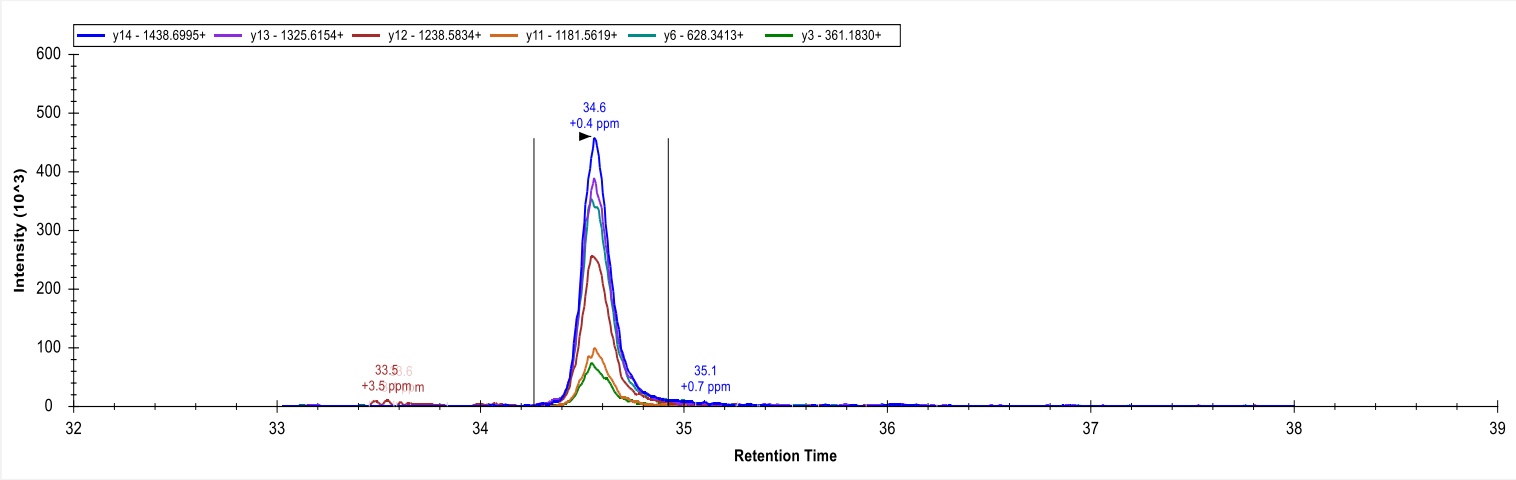
DISCOVERY RESULTS



- Carbonic anhydrase, actin (cytoplasmic), and fibrinogen had the same trends in horses with relevant time points
- Fibronectin had an opposite trend than the previous three proteins
- Average abundance of each protein in master horse sera pool shown

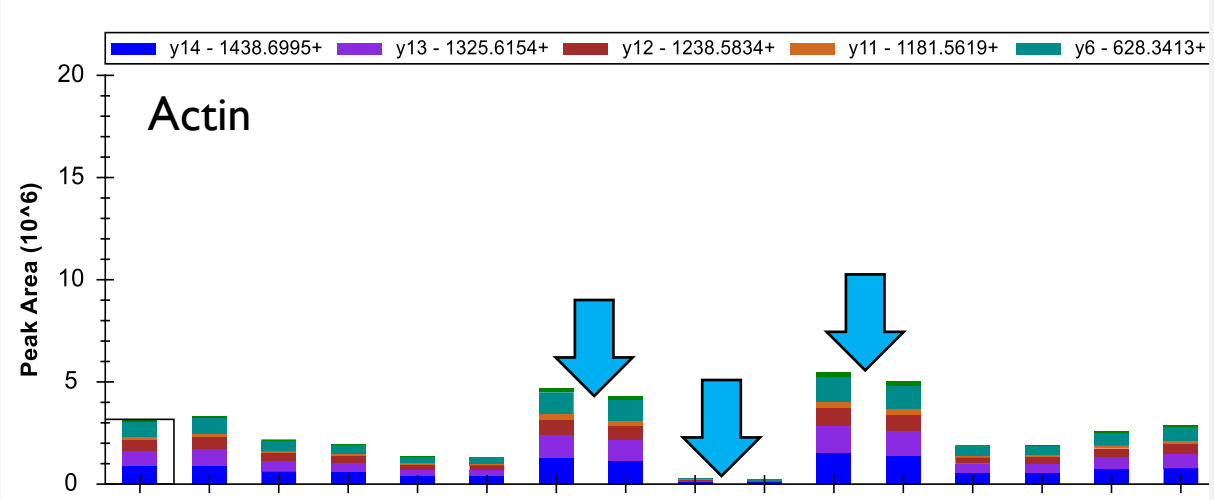
TARGETED RESULTS

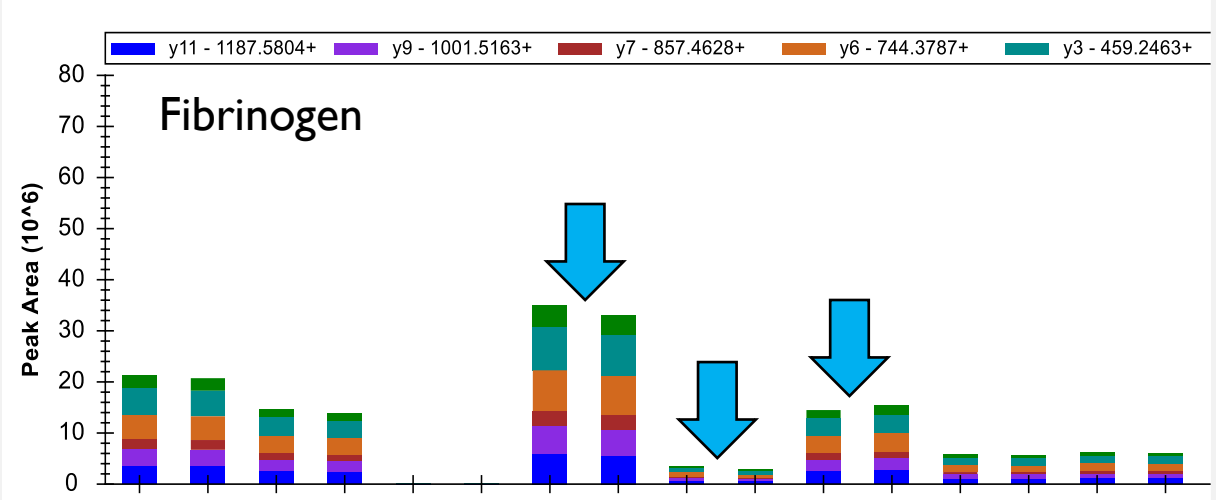
- Validated protein biomarkers that were originally identified in the discovery phase:
  - Actin
  - Carbonic Anhydrase
  - Fibrinogen
  - Fibronectin

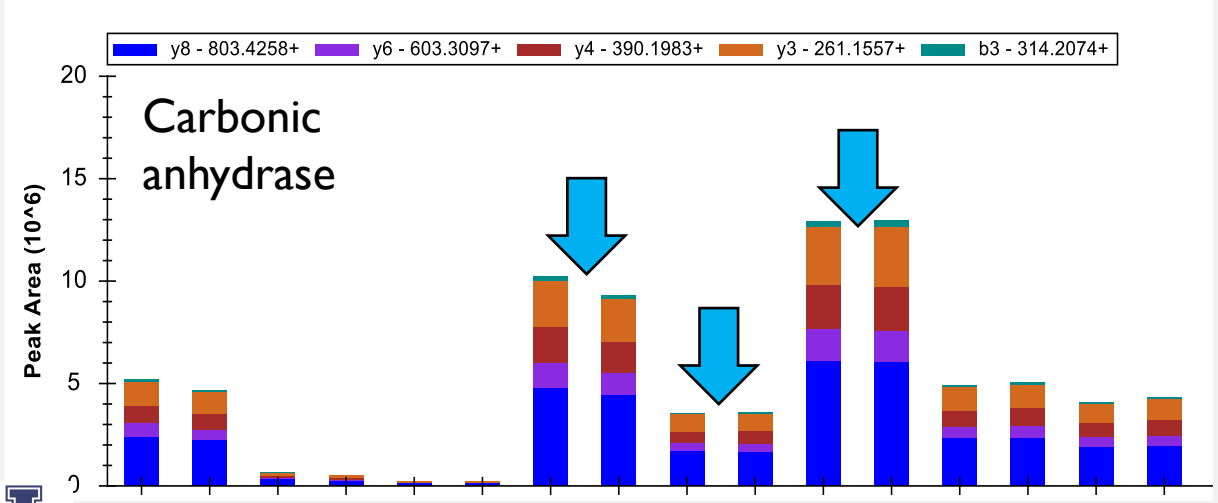


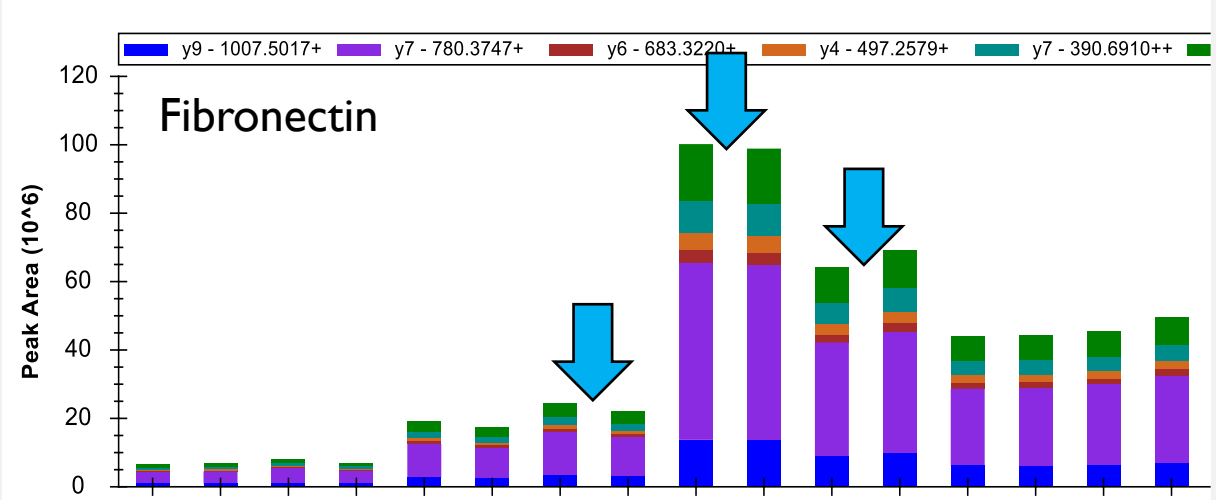
Skyline

SKYLINE DATA ANALYSIS



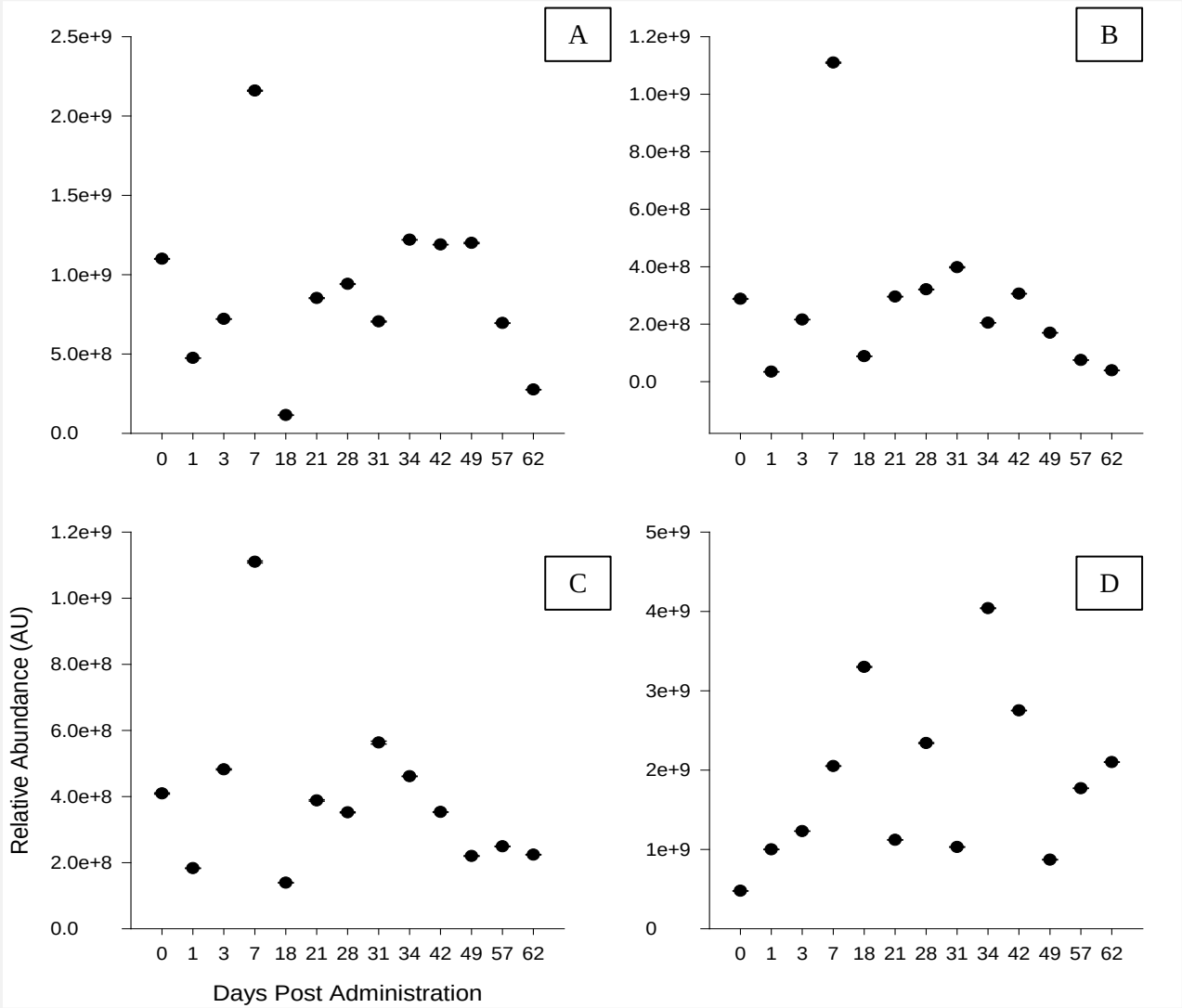






TARGETED RESULTS

- Abundance and standard error of each protein biomarker from day 0 to day 62
- Standard error bars are not visible due to low range
- Abundances are 2-log higher than those in discovery phase



Thank you for your attention

UK Equine Analytical Chemistry Lab

- Dr Abigail Burrows

STONESTREET Farm

- Barbara Banke
- Lesley Howard
- Gemma Freeman

Thermo Scientific

- Dr Amanda Lee
- Dr Mike Senko
- Dr Shannon Eliuk

QUESTIONS?